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Description

This invention relates to the field of clinical controls useful for calibrating clinical chemistry analyzers and in manual testing methods, as well as clinical standards and reagents. In addition, it relates to methods for producing stabilized liquid clinical control, standards, and reagents.

Chemistry analyzers occupy a preeminent position in the clinical environment, providing significant data regarding the diagnosis and treatment of patient illnesses. Accordingly, there has been a concerted effort by many investigators to develop automated and manual methods for the determination and qualification of constituents in body fluids such as acid phosphatase, alanine aminotransferase, albumin, aldolase, alkaline phosphatase, alpha-hydroxybutyrate dehydrogenase, amylase, aspartate aminotransferase, bicarbonate, direct bilirubin, total bilirubin, blood urea nitrogen, total calcium, carbon dioxide, chloride, total cholesterol, cholinesterase, cortisol, creatine kinase, creatine, digoxin, gamma glutamyl-transferase, globulin, glucose, HDL-cholesterol, iron, lactate dehydrogenase-1, lactate, osmolality, phenylalanine, phosphorus, potassium, salicylate, sodium, T3, T3 uptake, T4, total iron binding capacity, triglycerides and uric acid.

Both the manual and automated methods available for each one of the above analytes require either controls or reagents whereby the procedures and other variable parameters of the clinical chemistry analyzers may be checked to ensure accuracy of the testing method or instrument.

Conventional attempts to prolong the stability of chemistry controls and related reagents include reduction of the controls to a dry format which is typically refrigerated at approximately 4° C. Often, the dry format is made by lyophilization rather than spray drying as the former operates at far lower temperatures than the latter. Heat aggravates degradation of analytes and proteins in general and thus, cold processes such as freeze concentration and freeze drying, have been preferred. Although stability in a lyophilized state is enhanced over that associated with the aqueous format, significant losses in constituent activity levels of known chemistry controls are incurred.

One attempt at a freeze-stable liquid blood control standard is disclosed in U.S. Patent No. 4,199,471 to A.L. Louderback, et al. The standard comprises a sealed receptacle containing specifically treated red cells and a gaseous head space at least equal to about the volume of the red cells. The special treatment comprises thoroughly washing and separating the red cells from the plasma components and mild treatment with aldehyde, slow admixture and lower aliphatic diol or triol, and retention in a buffered solution. The special treatment optionally includes treating at least a portion of the red cells with carbon monoxide. The head space comprises from 0-15% CO_2, 0-25% O_2 and the balance N_2 and/or inert gas.

A different approach to stabilizing liquid clinical controls is disclosed in U.S. Patent 4,121,905 to Jonas Maurukus. Biological reference materials are lyophilized rapidly at -20 to -30° C, then reconstituted in a medium having 20 - 50% by weight of an alkylene polyol. The claimed stability is 4 to 5 weeks at 2 to 8° C.

The present invention provides a stable liquid clinical chemistry control that can be used either in a multiparameter format or in specific analyte formats. It also provides for clinical chemistry reagents which have improved stability at refrigerated temperatures.

More particularly, the liquid control comprises a storage pouch made with a water and oxygen impermeable material which is at least partially filled with either a clinical chemistry liquid control or reagent and an inert gas. The pouch has a reservoir for holding the liquid, and a filling inlet and a dispensing outlet which are heat-sealed and connected to the reservoir. For the purpose of this specification, the reservoir can have more than one filling inlet and dispensing outlet. Also, the reservoir can have more than one chamber such that the pouch as a whole holds a combination of materials. The chamber contents can be mixed either internally, by rupturing the dividing wall between the chambers, or externally, by having the chambers connected either to separate dispensing outlets or a single dispensing outlet.

The present pouch offers a variety of applications that can be tailored to the user. In one configuration, storable unit-dose liquid controls can be made which do not require remixing and measurement. A stable, ready to use control is available simply by tearing open the dispensing outlet and pouring out the pre-measured contents. Alternatively, the pouch can be filled with multiple doses and be provided with a folding flap at the bottom which permits the pouch to remain upright. Finally, if liquid reagents are used, the pouch can be filled only partially so as to permit additional fluids to be added, perhaps by pipetting, and mixed in the pouch.

Referring to the accompanying illustrative drawings:

Figure 1 is an overhead view of the present liquid control.

Figure 2 is a cross sectional view of a portion of the present liquid control.

The liquid control and reagent products of the present invention are useful for manual and automated

methods in chemistry analysis and, in particular, may be used with multichannel chemistry analyzers such as the ACA™ from DuPont or the SMAC™ from Technicon. The liquid control material is a patient-like sample characterized by a range of values for each constituent, enzyme and analyte found therein. For example, the liquid control, reagent or standard may comprise a multi-parameter control, an isoenzyme control, a hormone control or a coagulation control. The closer the control material simulates a patient's sample in providing all unknowns at their proper levels and in appearance (i.e., optical clarity and the like), the more useful it is. Similarly, the longer it presents such characteristics, i.e., the greater the time period it can hold the activity level for each constituent in a stable fashion, the more valuable the control material.

An example of the liquid control components that can be added are listed as follows:

CONSTITUENT	UNITS	LEVEL I	LEVEL II	LEVEL III
ACETOMINOPHEN	mg/l	10	20	50
ALBUMIN	g/dl	4	6	8
ALK PHOS	U/L	60	100	250
ALT	U/L	30	60	100
AMYLASE	U	50	100	300
AST	U/L	30	60	100
DIRECT BILIRUBIN	mg/dl	0.25	--	2
TOTAL BILIRUBIN	mg/dl	1	2	6
CALCIUM	mg/dl	8	10	12
CALCIUM, IONIZED	mEq/L	2	2.5	4
CARBON DIOXIDE	mEq/L	10	20	30
CHLORIDE	mEq/L	80	100	120
CHLORAMPHENICOL	µg/ml	50	75	100
CHOLESTEROL	mg/dl	60	120	240
CREATININE	mg/dl	1	2	6
CORTISOL	µg/dl	50	120	350
DIGOXIN	ng/ml	5	10	35
ETOH	mg/dl	50	100	150
GENTAMICIN	µg/ml	2	5	10
GLUCOSE	mg/dl	50	110	250
GGT	U/L	25	50	150
HBDH	U/L	100	200	450
HDL CHOLESTEROL	mg/dl	80	100	150
IRON	µg/dl	50	100	250

CONSTITUENT	UNITS	LEVEL I	LEVEL II	LEVEL III
LACTIC ACID	mg/dl	0.5	2.0	5.0
LD	U/L	60	120	350
LIPASE	U	10	25	50
LITHIUM	mEq/L	0	1	2
MAGNESIUM	mg/dl	1	2	4
MAGNESIUM	mg/dl	1	2	4
PHOSPHOLIPIDS	mg/dl	50	100	200
PHOSPHOROUS	mg/dl	2.5	4	8
POTASSIUM	mEq/L	2.5	4.5	6
SALICYLATE	mg/dl	2	5	10
SODIUM	mEq/L	120	140	150
T3	ng/dl	50	100	300
T4	µg/dl	2.5	7	14
TOBRAMYCIN	µg/ml	10	5	2
TOTAL LIPIDS	mg/dl	200	400	900
TOTAL PROTEIN	g/dl	4	6	8
BUN	mg/dl	10	20	45
URIC ACID*	mg/dl	3	6	10
VITAMIN B12	pg/ml	50	400	1000

* Both human and animal sources can be used.

As in the above example, preferably the controls and reagents have a combination of anaerobic bacteriacide added.

A preferred embodiment of the claimed control can be described best by reference to Figures 1 and 2. The general configuration of the control is a storage pouch 10 having a reservoir 12 with an arcuate bottom designed to contain a single unit-dose of liquid clinical control and a combined filling inlet/dispensing outlet 14. After a combination of inert gas (such as argon, krypton, neon, or nitrogen) and control is poured into the reservoir, the end of the inlet/outlet is sealed (16), preferably at ambient pressure.

If a multi-chambered reservoir is desired, the walls of the reservoir can be sealed to form a chamber dividing wall. If heat sealed the chamber will remain separate, however, if cold-roller sealed, then a burstable design is possible whereby one can gently squeeze the pouch, rupture the dividing wall, and thus mix the contents of the separate chambers.

The flexible pouch is made of a three layer laminate 20. The inner layer 22, which surrounds the reservoir 12, is made of a heat sealable water barrier, inert to the control liquid. A suitable material would be 25,4 µm (1 mil) polyethylene film. The middle layer 24 is an oxygen barrier, preferably 50,8 µm (2 mil) aluminum foil. The outer laminate layer is a puncture-resistant protection barrier that stands up to the physical stresses of transport and storage in bulk. A suitable material for this is 25,4 µm (1 mil) polyester film. The laminate can be formed by conventional means such as reverse gravure printing. Equivalent substitute materials would be known to the art.

The benefits in using the present pouch packaging for liquid controls and reagents are unexpected for several reasons. First, many of the biological components in controls and reagents are highly susceptible to denaturation and degradation at elevated temperatures, especially the 176° C (350° F) level needed to heat seal the above laminate. However, by adding the control and an inert gas under pressure substantially at

the same time, the control is never exposed to temperatures above 40° C. Also, the inert gas drives out ambient O_2 such that the dissolved pO_2 sample levels drop from $21,328 * 10^3$ to $1,333 - 2,666 * 10^3$ Pa (from 160 to 10 - 20 torr). Unlike oxygen permeable containers, the present pouch does not allow easy oxygen mixing, and thus, components such as bilirubin, uric acid and sulfhydryl enzymes, which would oxidize readily at clinical pH (7.4) are stable for long periods of time.

The elimination of any light transmitted to the contents of the pouch prevents photo-chemical processes from degrading any of the analytes which are present in the formulation.

EXAMPLE 1

A useful clinical chemistry control combines a series of analytes together in such a way that the sum of the analytes mimic a patient's specimen under ordinary laboratory conditions. Each of these individual analytes are measurable by any number of chemical, electrical, and immunological methods. Therefore, with an increasing number of analytes in a particular control material, one would expect increasing interferences with many of these methods. Whole human serum, the liquid portion of the blood separated from the clot, is the sample of choice for many of the routine chemistry tests run in the hospital laboratory. The analytes which have clinical significance in this serum are generally stable for use within a one-day period after the serum has been separated from the red blood cells. These analytes degrade with time by a series of destructive mechanisms, i.e., heat, oxidation, light. Thus, ordinary human serum of defribrinated plasma is unsuitable as a base material for a liquid control.

In order to ensure that all analytes are stable, it is necessary that the base protein be free of most of the unstable analytes targeted for use in the control. It has been useful for us to start with a base of either fraction 5 albumin or serum which has been "stripped" of any of these labile components. In the case of albumin solutions, a buffer is added to control the pH of the control material during the stability period. A series of antimicrobial agents are added to minimize the growth of microorganisms in the control medium. To this matrix, analytes are added as stabilized materials to this stabilized matrix -- each designed to minimize interactions with other analytes. For example LDH is added as a purified preparation from chicken heart which is far more stable than normal serum LDH.

To minimize the oxidation of all components which are added to the control, the matrix is maintained at a very low level of dissolved oxygen during the preparation and filling of the control. This is accomplished by maintaining an inert gas presence in intimate contact with the product during processing.

The presence of the inert gas and the exclusion of additional oxygen due to the composition of the pouch, minimizes the changes in analyte values during any subsequent freezing and thawing of the material. Under ordinary conditions many analytes lose significant activity if they are frozen and thawed.

EXAMPLE 2

It is useful, both in the doctor's office and the hospital laboratory, to have a calibrator to verify the effectiveness of the urine "dipsticks" which are used routinely in urinalysis. A liquid control material has been formulated where the constituents which are analyzed using current dipstick methods are added to a stabilized human urine preparation. The present pouch is filled with this preparation in the presence of argon. The pouch has suitable dimensions for inserting a dipstick directly into the pouch to come into contact with the control material. This single-use control concept would be eminently suitable for a doctor's office as well as a large hospital laboratory.

EXAMPLE 3

The present pouch has been designed with multiple compartments to contain reagents which would be incompatible for extended storage periods. These compartments would be separated by a heat seal which could be ruptured, causing the two reagents to mix in a single compartment of the pouch. A patient's sample could be added to the contents of the pouch by means of pipetting, and the entire contents of the pouch aspirated into a spectrophotometer or other instrument to quantitate the analyte being measured.

The final result is an unexpected long stability period. At refrigerated temperatures of 5 - 8° C, controls and reagents are stable for 2 - 8 months, whereas when frozen at -10° C, the stability shown by present data supports predictions of stability from 3 to 5 years.

Claims

1. A stable protein-based liquid control, reagent or standard characterised in that it comprises:
   (a) a storage pouch (10) having a reservoir (12) and connected to the reservoir, at least one heat-sealed filling inlet (14) and at least one heat-sealed dispensing outlet (14);
   (b) the pouch being made of a water and oxygen impermeable material; and
   (c) the reservoir being at least partially filled with a protein-based control, reagent or standard liquid and an inert gas.

2. A control, reagent or standard as claimed in claim 1 wherein the filling inlet also serves as the dispensing outlet.

3. A control, reagent or standard as claimed in claim 1 or claim 2 wherein the reservoir has at least two chambers separated by a dividing wall.

4. A control, reagent or standard as claimed in any of claims 1 to 3 wherein the pouch is at least partially made of a laminate comprising:
   (a) an inner layer which is heat-sealable, a water barrier, and inert with respect to the control, reagent or standard liquid;
   (b) a middle layer which is an oxygen barrier; and
   (c) an outer layer which is a puncture-resistant protection barrier.

5. A control, reagent or standard as claimed in claim 4 wherein the laminate layers comprise polyethylene film as the inner layer, aluminum foil as the middle layer, and polyester film as the outer layer.

6. A control, reagent or standard as claimed in any of claims 1 to 5 wherein the inert gas comprises argon, krypton, nitrogen or neon.

7. A control, reagent or standard as claimed in any of claims 1 to 6 wherein the liquid control comprises a multi-parameter control, an isoenzyme control, a hormone control or a coagulation control.

Revendications

1. Un témoin, réactif ou étalon liquide stable à base de protéine(s) caractérisé en ce qu'il comprend :
   (a) une poche de stockage (10) comportant un réservoir (12) et, reliés au réservoir, au moins un passage d'entrée de remplissage (14) scellé à chaud et au moins un passage de sortie de distribution (14) scellé à chaud;
   (b) la poche étant formée d'un matériau imperméable à l'eau et à l'oxygène; et
   (c) le réservoir étant au moins partiellement rempli d'un liquide témoin, réactif ou étalon à base de protéine(s) et d'un gaz inerte.

2. Un témoin, réactif ou étalon selon la revendication 1 dans lequel le passage d'entrée de remplissage sert aussi de passage de sortie de distribution.

3. Un témoin, réactif ou étalon selon la revendication 1 ou la revendication 2 dans lequel le réservoir comporte au moins deux chambres séparées par une cloison de séparation.

4. Un témoin, réactif ou étalon selon l'une quelconque des revendications 1 à 3 dans lequel la poche est au moins partiellement formée d'un stratifié comprenant :
   (a) une couche interne qui est scellable à chaud, imperméable à l'eau et inerte vis-à-vis du liquide témoin, réactif ou étalon;
   (b) une couche intermédiaire qui est imperméable à l'oxygène; et
   (c) une couche externe résistante à la perforation qui forme barrière de protection.

5. Un témoin, réactif ou étalon selon la revendication 4 dans lequel les couches du stratifié comprennent un film de polyéthylène en tant que couche interne, une feuille d'aluminium en tant que couche intermédiaire et un film de polyester en tant que couche externe.

6. Un témoin, réactif ou étalon selon l'une quelconque des revendications 1 à 5 dans lequel le gaz inerte comprend de l'argon, du krypton, de l'azote ou du néon.

7. Un témoin, réactif ou étalon selon l'une quelconque des revendications 1 à 6 dans lequel le témoin liquide comprend un témoin multiparamétrique, un témoin isoenzymatique, un témoin hormonal ou un témoin de coagulation.

Patentansprüche

1. Stabiles, flüssiges Kontroll-, Reagenz- oder Standardmittel auf Proteinbasis, gekennzeichnet durch
   a) eine Vorratstasche (10) mit einem Reservoir (12) und wenigstens einem verschweißten Einfülleinlaß (14) und wenigstens einem verschweißten Abgabeauslaß (14), die mit dem Reservoir verbunden sind;
   b) wobei die Tasche aus einem wasser- und sauerstoffundurchlässigen Material besteht; und
   c) wobei das Reservoir wenigstens teilweise mit einer Kontroll-, Reagenz- oder Standardflüssigkeit auf Proteinbasis und einem inerten Gas gefüllt ist.

2. Kontroll-, Reagenz- oder Standardmittel nach Anspruch 1, bei dem der Einfülleinlaß auch als Abgabeauslaß dient.

3. Kontroll-, Reagenz- oder Standardmittel nach Anspruch 1 oder 2, bei dem das Reservoir wenigstens zwei Kammern besitzt, die durch eine Trennwand separiert sind.

4. Kontroll-, Reagenz- oder Standardmittel nach einem der Ansprüche 1 - 3, bei dem die Tasche wenigstens teilweise aus einem Laminat hergestellt ist, bestehend aus:
   a) einer Innenschicht, die verschweißbar ist, eine Wassersperre bildet und bezüglich der Kontroll-, Reagenz- oder Standardflüssigkeit inert ist;
   b) einer Mittelschicht, die eine Sauerstoffsperre darstellt; und
   c) eine Außenschicht, die eine punktionssichere Schutzsperre ist.

5. Kontroll-, Reagenz- oder Standardmittel nach Anspruch 4, bei dem die Laminatschichten einen Polyethylenfilm als Innenschicht, eine Aluminiumfolie als Mittelschicht und einen Polyesterfilm als Außenschicht aufweisen.

6. Kontroll-, Reagenz- oder Standardmittel nach einem der Ansprüche 1 - 5, bei dem das inerte Gas Argon, Krypton, Stickstoff oder Neon aufweist.

7. Kontroll-, Reagenz- oder Standardmittel nach einem der Ansprüche 1 - 6, bei dem das flüssige Kontrollmittel ein Mehrparameterkontrollmittel, ein Isoenzymkontrollmittel, ein Hormonkontrollmittel oder ein Koagulationskontrollmittel aufweist.

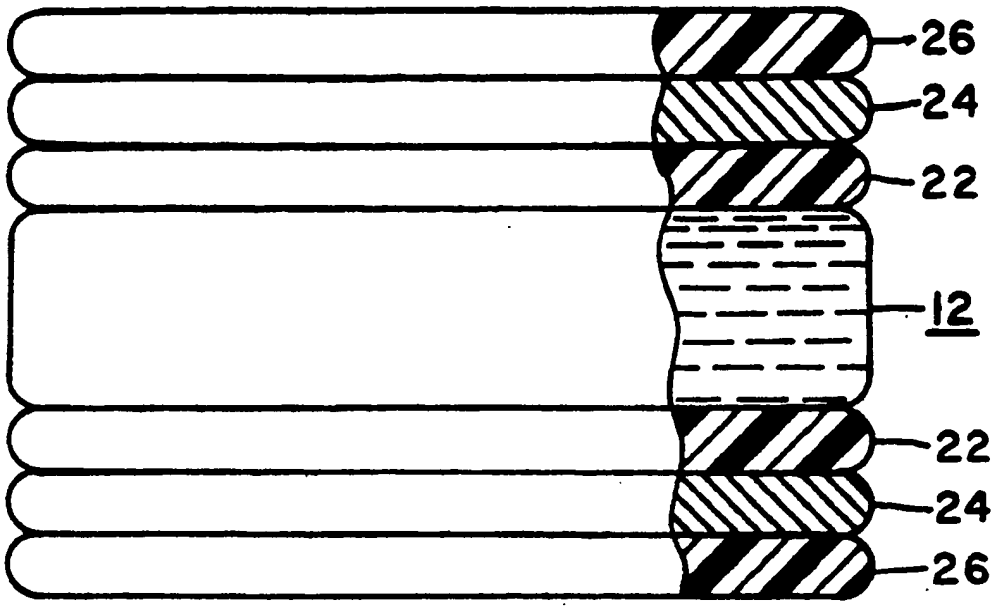

Fig. 1

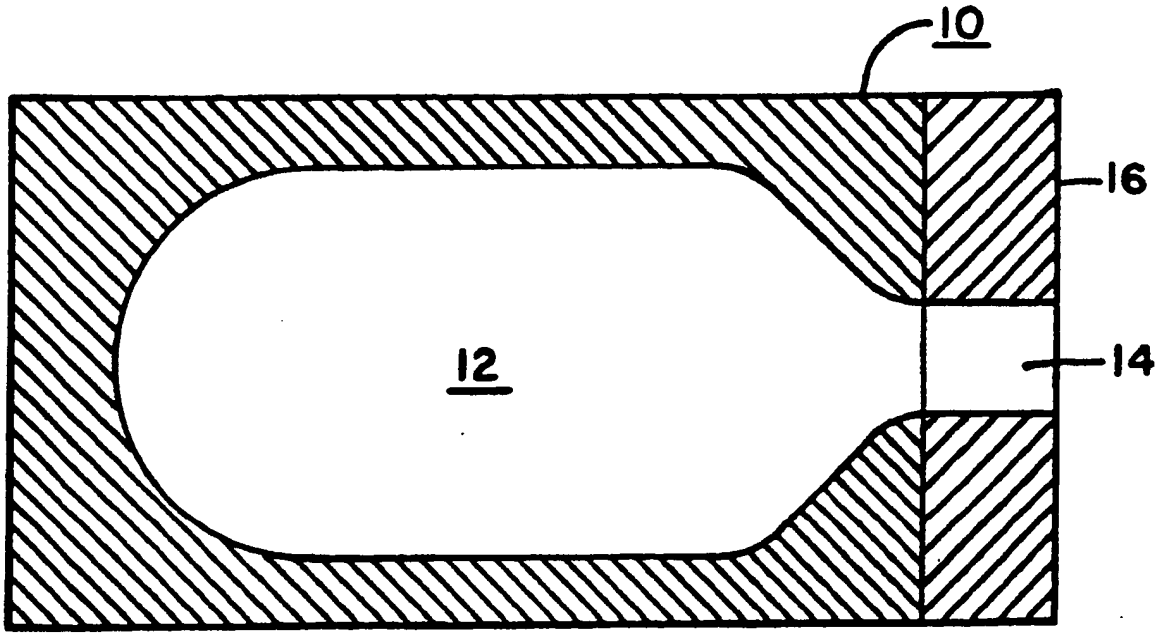

Fig. 2